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In order to focus this prosecution on the method embodiments, Applicants have elected to cancel claims directed to other embodiments, such as those concerning adenovirus compositions *per se*, and those concerning host cells adapted for serum-free growth. Applicants reserve the right to represent the subject matter of these claims in later continuing applications.

In the current claims, the phrase "low perfusion rate" is no longer employed. Instead, in certain claims (*e.g.*, claim 70) Applicants employ the use of the glucose level to define the perfusion rate (*e.g.*, perfusion sufficient to maintain glucose at a level of less than 2.0 g/L). Support for the amendment of claim 70 in this regard can be found in Example 2 and Table 5, pages 80-81, of the specification.

It is also of note that the "perfusing" step has been separated from the "growing" step in order to clarify that the perfusion need not be continuous during cell culturing (see, *e.g.*, page 26, lines 15-22).

Amendments to claim 22 (and in new claims 110 and 118) are an attempt to clarify the nature of the invention of those claims, which concerns the surprising finding that very significant purification can be achieved with only a single chromatography step, without the concomitant loss of yield associated with the use of multiple chromatographic separations. The phrasing of these claims is specifically intended to allow the use of other purification methods (*i.e.*, non-chromatographic methods) in addition to the single chromatographic step and still be within the scope of these claims.

New claims 101 and 118 make reference to the use of lysing techniques "other than freeze-thaw." Support for this amendment can be found in section 3, pages 29-36, of the specification, wherein numerous techniques for achieving lysis other than through the use of

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freeze-thaw are set forth. As noted in Table 1, page 30, freeze-thaw is not recommended for large scale manufacturing and thus has been excluded from the scope of these claims.

New claims 119 and 120 were added to clarify that indeed the term “perfusion” is intended to cover both continuous and fed-batch perfusion processes. Again, the use of the term “continuous perfusion” in claim 120 is not intended to imply that the perfusion is somehow continuous throughout the cell growth process; rather, “continuous perfusion” is used in this claim simply to distinguish this type of perfusion (where during the perfusion, the introduction of media is more or less continuous) from a fed-batch process. Support for these claims can be found in the specification at page 20-21.

The new claims were added to focus on specific embodiments that are believed to be clearly patentable over the art, principally embodiments involving the use of a single chromatography step (*e.g.*, claims 110 and 118). Support for these claims can be found, for example, in the originally filed claims. Some secondary claims are also included which are essentially repositioning of subject matter from the originally filed claims.

## **II. Rejections Under 35 U.S.C. §112, Second Paragraph**

Claims 1-42, 61-63 and 69 were rejected under 35 USC §112, second paragraph, as being indefinite.

Applicants will deal first with the “low perfusion rate” issue as it relates to the pending claims. As noted above, Applicants have amended the claims to clarify what was intended by the term “low perfusion rate.” Applicants chose to define this term by reference to a perfusion rate that will maintain the glucose level in the culture medium during times of perfusion at a range of

less than 2.0 g/L (claim 70), or more preferably, between 0.7 g/L and less than 2.0 g/L. It is to be noted that the glucose level need not always be within this range during adenovirus production. In fact, it is typically the case that cells will be grown in culture for a period of time prior to or even after periods of perfusion. Prior to perfusion, the glucose level will typically start at a somewhat higher level (*e.g.*, around 5 or so g/L) and during time of culture the glucose level will slowly drop as the glucose is metabolized by the cells. However, during periods of perfusion, it is preferred that a perfusion rate be chosen that will maintain a glucose level of between 0.7 and 2.0 g/ml and even more preferably between about 0.7 and about 1.7 g/l.

### **III. Claimed Objections Under 35 CFR §1.75(c)**

Applicants believe that no response to the objection with respect to claim 57 is required in light of the fact that this claim is no longer pending. The issue with respect to claim 53 has been addressed in that this claim no depends from claim 20.

### **IV. Rejections Under 35 USC §102**

The only claims subject to rejection under 35 USC §102 are claims 66, 67 and 69. Since none of these claims is currently pending, no response is believed to be required here.

### **V. Rejection of Claims 31-42, 51 and 65 under 35 USC §102(b)/103(a)**

Since claims 31-42, 51 and 65 are no longer pending, Applicants are not required to respond to this basis of rejection.

## **VI. Rejection of Claims Under 35 USC §103(a)**

The action has rejected certain claims as obvious over the combination of Huyge et al in view of Perrin et al, Garnier et al, Nadeau et al and any of Fanget et al, Trepanier et al, or Payment et al. The Examiner appears to take the position that the Huyge et al reference discloses a method for producing an adenovirus that is generally consistent with Applicants' method, but agrees that Huyge does not disclose perfusion, concentration and buffer exchange. The Examiner concludes that perfusion cultures have long been used for large-scaled growth of cells for virus production, referring to the secondary articles.

In response, Applicants respectfully submit that none of the cited references, alone or in combination, teach or suggest the invention of the pending claims. Of the currently pending claims, those that focus on the perfusion issue are claims 70-100. Applicants will thus focus on these claims in the context of the present rejection.

Claim 70 is directed to the surprising finding that the use of a low perfusion rate during a selected stage of host cell growth resulted in dramatic improvements in the purity of virus material ultimately obtained. This fact is shown in Example 2 on pages 80-81 of the specification, as well as Figure 1. In these studies it is surprisingly shown that a low perfusion rate, as compared to a high perfusion rate (rates that maintain a glucose level of 2.0 g/L or higher), provides a very high degree of separation of peaks upon subsequent chromatographic purification. In fact, Figure 1 shows that a very well separated virus peak (retention time 9.39 minutes) was produced from lysates made using low perfusion rates. It was further found that virus with a very high degree of purity and biological activity could be obtained after ion exchange chromatographic purification of the lysate produced using low perfusion rate (see

Figure 1B). In stark contrast to this, no separated virus peak in the retention time of 9.39 minutes was observed from lysate produced using a high perfusion rate (i.e., a rate that maintained the glucose concentration at greater than or equal to 2.0 g/L during times of perfusion).

This suggests that contaminants which have the same elution profile as the virus were produced under high perfusion rate. It is speculated that the contaminants are related to the increased extracellular matrix protein production under high perfusion rate (high cell feeding) from the producer cells. The use of a low perfusion rate therefore produces easy-to-purify crude product and also offers a more cost-effective production due to the reduced media consumption.

Applicants have reviewed the references relied upon by the Examiner and have been unable to identify any teaching or suggestion relevant to the use of a low perfusion rate for producing adenovirus, and certainly no suggestion that the use of a low perfusion rate would be advantageous.

The Perrin *et al.* reference (C25) relates to a rabies virus system quite distinct from adenovirus. The fact that Perrin et al. teaches perfusion in the context of rabies virus production would in no way motivate the use of perfusion in the context of adenovirus production. The reason for this is that rabies virus is an enveloped “budding” RNA-based rhabdovirus whereas adenovirus is a DNA capsid based non-enveloped virus of an entirely different viral family – these viruses infect and grow differently and indeed replicate differently. Moreover, adenovirus is a very fragile virus as compared to viruses like rabies virus and one would not expect that it could be handled in any where near the same manner as rabies virus. Therefore, there would be no *a priori* expectations that the optimal conditions in one system would be optimal or even functional in another system.

More importantly, Perrin *et al.* says nothing about advantages in terms of ease of purification and purity that one might obtain through the use of a low rather than high perfusion rate. This in itself is strong evidence of non-obviousness, and a finding that is in no way taught or suggested by Perrin *et al.*

Furthermore, it appears as though the perfusion rate disclosed by Perrin *et al.* is a fairly high rate in that it involves an exchange of 144% of the reactor volume/day (page 1245, col. 2). From the description provided, it would appear that this 144% exchange would correspond to maintenance of a glucose level at significantly greater than 2/gL based on the present inventor's experience in the preferred systems which they employ that a 100% volume exchange per day will maintain a glucose level of approximately 1.5/gL.

While Applicants agree with the Examiner's position that fed-batch systems are one type of perfusion system (see Specification, pages 20-21), Nadeau *et al.* is similarly not considered relevant for the reason that it fails to teach that perfusion at low perfusion rates (such as through a fed batch process) can provide a more readily purifiable adenovirus product. Nadeau *et al.* does experiments with maintaining glucose levels within certain defined ranges for the purposes of improving recombinant protein production, which is unrelated to Applicants' invention which concerns producing recombinant adenovirus itself – the Applicants claims are indeed not related in any way to recombinant protein production. Indeed, there is no teaching or suggestion in Nadeau *et al.* that maintaining glucose levels within a defined range would render the resultant adenovirus more readily isolated and capable of being more easily purified to a high degree of purity.

Similarly, the Garnier *et al.* reference does not appear to teach or suggest that low perfusion rates, characterized as rates that will maintain a glucose level of less than 2 g/L, will provide particular advantages in the production of highly purified adenovirus. Indeed, Garnier *et al.* relates to improved recombinant protein production and says nothing about the effects of fed batch on adenovirus particle production and says nothing about whether fed batch would have an advantageous effect on the ability to provide highly purified adenoviral particles.

Furthermore, Garnier *et al.* shows in Fig. 2(a) that when glucose levels fall below about 15 mM (2.7 g glucose/L) that the rate of increase in active recombinant protein production comes to a plateau. Thus, one of skill would endeavor in light of Garnier *et al.* to maintain the glucose level at at least 2.7 g glucose/L. This is consistent with the fact that Garnier *et al.* teaches medium replacement using a 2.0 g glucose/L stock (page 154, "Conclusion"). Thus, overall the Garnier *et al.* reference suggests that glucose limitation has a negative effect on protein production and recommends glucose supplementation.

For the foregoing reasons, Applicants respectfully submit that the low perfusion rate claims are patentable over the art.

## **VII. Rejection of Claims 17-19 and 43-50 Under 35 USC §103(a)**

The Action next rejects claims 17-19 and 43-50 as obvious over Huyge *et al.* reference in view of Perrin *et al.*, Garner *et al.*, Nadeau *et al.* and Fanget *et al.*, Trepanier *et al.* or Payment *et al.*, all of these further in view of Graham *et al.* (C7). The Examiner reasserts the basic rejection set forth above, and adds in Graham *et al.* for the proposition of teaching the use of a 5% sodium

deoxycholate which, according to the Examiner, can be used to disrupt cells without disrupting adenovirus virions.

In responding to this rejection, Applicants submit that the relevant claims are those directed to lysing by methods other than freeze-thaw, represented by claims 101-109.

In response, Applicants respectfully point that the Graham et al. article employs the “freeze-thaw” technique for adenovirus purification and does not teach a technique that is detergent based as suggested by the Examiner. For example, in the paragraph at the bottom of page 118 it is indicated that the adenovirus stocks are stored at -70 degrees centigrade until needed, followed by thawing the frozen virus stock as discussed at the top of page 119. Sodium deoxycholate merely serves to solubilize already disrupted cell membranes and is not typically a detergent used in disrupting membranes *per se*. Indeed, sodium deoxycholate is an ionic detergent and would thus be contraindicated for use in concentrations high enough to effect cell lysis where purification of adenovirus was the ultimate goal. For example, Table 1 of Applicants’ specification, which concerns methods used for cell lysis, makes reference to the use of “non-ionic” detergents. Thus, one of skill in the art reviewing the Graham et al. article would merely conclude that Graham was teaching the well-known freeze-thaw technique for adenovirus production. This is consistent with the teaching of, for example, Huyge et al., which also teaches the freeze-thaw method.

Applicants note that claim 101 requires the use of a lysing technique other than freeze-thaw, and thus distinguish freeze-thaw technology.



### **VIII. Rejection of Claims 52-58, 60-64 Under §103(a) and Claim 68 Under §102(a)**

In light of the fact that Applicants are not proceeding with claims that rely solely on the serum-free culture aspects of the present invention, it is believed that no response to this rejection is required. (While claims 53-56 are pending, they now depend from previously allowable claim 20, which is separately patentable on the basis of the combination use of perfusion in combination with autolysis – see below.)

### **IX. Claim 20 and Those Depending Therefrom are Allowable**

The Examiner has previously indicated that claim 20 is free of the prior art. The only pending rejection as to claim 20 was under §112, second paragraph, on the basis of the “low perfusion rate” language. Claim 20 has been placed into independent format, and no longer recites a low perfusion rate.

### **X. New Claim 118**

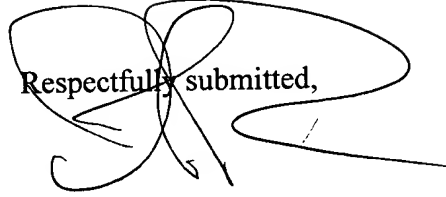
New claim 118 combines aspects of several of the claim groups, including the use of perfusion, lysing using techniques other than freeze-thaw, and use of a single chromatography step. It is submitted that this claim particularly far removed from the prior art.

### **XI. Conclusion**

It is submitted that the present case is in condition for allowance, and such is earnestly solicited. If the Examiner has any questions, comments or suggestions, a telephone call to the

undersigned applicants' representative is requested.

Respectfully submitted,

A large, stylized handwritten signature in black ink, appearing to be 'D. Parker', written over the words 'Respectfully submitted,'.

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